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Purification and Properties of a Prokaryote Type Glutamine Synthetase from the Bialaphos Producer *Streptomyces hygroscopicus* SF1293

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A prokaryote type glutamine synthetase (GS) was purified from a bialaphos (BA)-producing organism, *Streptomyces hygroscopicus* SF1293 (SF1293). The GS (GS I) consisted of a 55,000 dalton subunit, and its N-terminal amino acid sequence was similar to that of *S. coelicolor* GS. GS I was highly sensitive to GS inhibitor phosphinothricin (PPT). An increase of GS activity was observed accompanied by BA accumulation.

GS (EC 6.3.1.2) catalysing the conversion of l-glutamic acid to l-glutamine in the presence of ammonia and ATP is essential for the biosynthesis of glutamine. SF1293 produces a herbicide BA (1, 2) and its structural component PPT is a strong GS inhibitor (3). Though SF1293 has a PPT detoxicating enzyme PAT (PPT acetyltransferase (4, 5) which acetylates the amino group of PPT, the organism is always exposed to PPT during BA production.

Considering the above, we have been interested in the change of GS activity during BA production. We also find the properties of SF1293 GS of interest because we have obtained the result that SF1293 has a gene (*gln* II) encoding a GS which resembles a eukaryote type enzyme GS II (6) with respect to its molecular weight and amino acid sequence.

The BA producer, SF1293, was obtained from the Meiji Seika Culture Collection. The cultivation conditions for BA production were as follows. The strain was precultured in 10 ml of S1 medium (5, 7) at 28°C for 1 d, and 1 ml of the seed culture was transferred to 30 ml of the production medium (5, 7). The temperature and agitation speed were 30°C and 220 rpm (rotary shaker), respectively.

Preparation of cell-free extracts was carried out as follows. Forty-grams of wet mycelium (100 ml culture broth) was suspended in 65 ml of cold Buffer I (20 mM Imidazole, 1 mM $MnCl_2$, pH 7.5), and disrupted by sonication on ice. The cell debris was removed by centrifugation (17,000 × *g*, for 15 min, at 4°C), and the supernatant was dialyzed against the cold Buffer I.

Formation of γ-glutamylhydroxamate from glutamate, hydroxylamine and ATP was assayed at 37°C, pH 7.55 to estimate GS activity, as described by Bender *et al.* (8). One unit of the enzyme was defined as the amount producing 1 μmol of γ-glutamylhydroxamate per min.

Quantitative analysis of BA in culture was carried out according to the method of Seto *et al.* (9) with the following modification. The culture broth was diluted with water, heated for 5 min in boiling water and centrifuged. The resulting supernatant was used for the assay.

N-Terminal amino acid analysis of GS was performed by Edman degradation using an automatic amino acid sequencer.

SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (10) with the following modifications. Samples were denatured in boiling water for 5 min in the presence of 1% SDS and 10% 2-mercaptoethanol before loading onto a 12% polyacrylamide gel. SDS-PAGE Standard Low (Bio-Rad) was used as protein size markers.

Purification of GS (I) was carried out by the method of Paress and Streicher (11) with modifications. Blue-Sepharose CL-6B (Pharmacia) was added into the cell-free extracts (12 units/mg protein) prepared from 500 ml of culture broth. The suspension was packed in a glass column after stirring overnight at 4°C. The column was washed with Buffer II (Buffer I + 1 M NaCl). GS (I) was adsorbed to Blue-Sepharose CL-6B under this condition. GS (I) was eluted with Buffer I containing 5 mM-ADP. Twenty ml of the active fractions (210 unit/mg protein; 17.5 fold, yield 10%) were collected. The enzyme solution was charged to a reverse phase HPLC column. A gradient of 0 to 100% CH_3CN was used to elute GS. Five ml of GS (I)-rich fraction was collected. Two mg of GS (I) was obtained. The purity of GS (I) was analysed by SDS gel electrophoresis, as shown in Fig. 1. Over 95% purity was obtained.

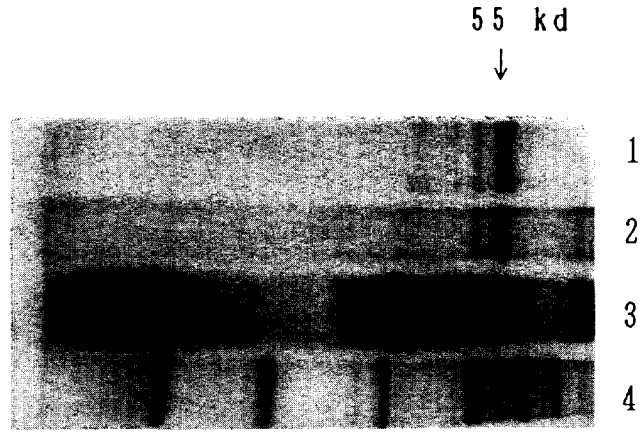

FIG. 1. SDS gel electrophoresis of GS I. The purity of GS I in each step of the purification was monitored by SDS gel electrophoresis. Lane 1, After the 2nd step (reverse-phase chromatography). Lane 2, After the 1st step (Blue-Sepharose CL-6B). Lane 3, Before purification (Cell-free extract). Lane 4, Standard proteins low (Bio-Rad).

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TABLE 1. N-Terminal amino acid sequences

GS	Amino acid number from N-terminal														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S. hygroscopicus SF1293 GS I	Met	Phe	Gln	Asn	Ala	Asp	Glu	Ala	Lys	?	Phe	Ile	Ala	Asp	Glu
S. coelicolor GS	Met	Phe	Gln	Asn	Ala	Asp	Asp	Val	Lys	Lys	Phe	Ile	Ala	Asp	Glu

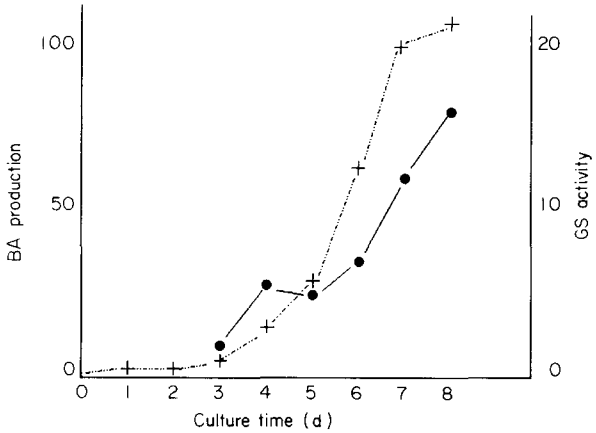

FIG. 2. Change of GS activity during BA production. Symbols: (●) and (×) show the GS activity (unit/mg protein) and BA production (% of the maximum concentration in the medium) in the cellfree extract, respectively.

SDS gel electrophoresis (Fig. 1) indicates that GS (I) consists of a 55 kilo dalton subunit as *S. coelicolor* GS (12). Table 1 shows N-terminal amino acid sequences of GS (I) and *S. coelicolor* GS. Both were the same except for the 7th and the 8th amino acids. GS (I) was sensitive to GS inhibitor PPT, *i.e.* 50% inhibition concentration of PPT was under 5 μM (data not shown). As shown in Fig. 2, the increase of GS activity in the cellfree extracts of SF1293 was accompanied by BA accumulation.

GS I may be a prokaryote type GS as *S. coelicolor* GS, judging from the data of the subunit molecular weight and its N-terminal amino acid sequence. This shows that *S. hygroscopicus* contains genes encoding at least two distinct GS isoforms, *i.e.* one is a prokaryote type GS I and another is a eukaryote type GS II.

Increase of GS activity during cultivation has not been observed in the case of *E. coli* (13) and *Streptomyces cattleya* (14). Though GS I was very sensitive to PPT, overproduction of GS I may play a role in a self resistance mechanism to PPT or BA. In fact, it has already been reported that high GS activity offered the host organism resistance to BA or PPT (15, 16).

Though GS II was overproduced in the transformants of *S.lividans* and SF1293 harboring *gln* II plasmid (6), we could not purify GS II from SF1293. The expression of GS II in SF1293 is not yet clear.

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